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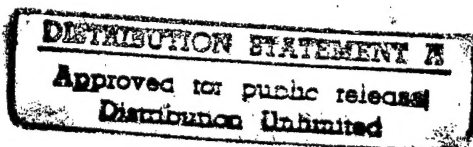
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ON METABOLISM

- USSR -

by Libor Bozar

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COMPLEXON -3 IN FOOD PRODUCTS AND ITS
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Libor Bozar in Voprosy Pitaniya (Problems of Nut-
rition), Vol. 19, No. 1, Moscow, 1960, pages
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In attempting to eliminate the negative effect of
metals or metal-containing enzymes in the nutrition industry,
efforts are being made to utilize the so-called complexons
(derivatives of alpha-aminopolycarbonic acids). These are
distinguished by their unusual complex-forming affinity
with various metals. The utilization of complexons -- mainly,
the bisodium salt of the ethylenediamine-tetracetic acid (com-
plexon-3) in food technology -- is based principally on the
complexide constants which increase in the order:



This property was utilized in 1948 in the elimination
of the catalytic oxidation of ascorbic acid by copper. It
was demonstrated in vitro (Jager, 1948) that it is possible
to eliminate, with the aid of complexon-3, the catalytic ef-
fect of copper in an ascorbic acid solution. On this basis,
complexon-3 was successfully employed as a stabilizer of
Vitamin-C (Erdey, 1950). Other authors also preserved
Vitamin-C solutions in a similar manner (Schulte and
Schillinger, 1952; Morse, 1953).

However, complexon-3 was not considered more effect-
ive than metaphosphoric acid solutions which already had
been used a long time ago. It was proven that though com-
plexon-3 blocks the metal cations present in the solution,
this material, nevertheless cannot inhibit the self-oxidation
of ascorbic acid.

Complexon-3 is used also in complexometric titration
of various cations (Pribil, 1957).

The stabilizing properties of complexon-3 in relation

to ascorbic acid make possible its wide utilization in the food industry. It has been demonstrated in extensive experiments (Licciardello, 1952) that the addition of small quantities of complexon-3 retards the disintegration of Vitamin C in orange juice. This author observed considerable preservation of vitamin C also in the manufacture of red bilberry garnishes. Similarly, in the technique of ordinary food preparation it was possible to preserve a large quantity of vitamin C in the presence of complexon-3 (Baranovic, 1957). Dishes prepared with the use of complexon-3 did not differ organoleptically from dishes prepared without this stabilizer.

Complexon-3 may also be used in the filtration of wine and other alcoholic and non-alcoholic beverages. It is known that in technical processes of this type one is concerned most frequently with the removal of the excessive content of trivalent iron. In wine refining up to present time, potassium ferricyanide has been used for this purpose, the potassium compound producing an intense precipitate of Prussian blue with the trivalent iron. It was proven (Krum and Fellers, 1952) that, the high complexity constant of the ferrous complex of complexon-3 resulting from its good solubility, permits one to easily substitute the filtration of wine by adding the needed quantity of complexon-3. According to other data (Farkas, 1957), the employment of this method (masking) may help in rationalizing production and the obtainment of better organoleptic indices of an annual output.

The binding between complexon-3 and copper or iron makes possible the utilization of complexons in technological practice, also, in blocking the undesirable manifestations of various enzymes, especially the redoxidase of copper or iron. The complex formation of complexon-3 in this sense was employed for the first time for the elimination of potato darkening. Thus, it was observed (Greigy and Smith, 1955) that potatoes do not become darker even after 24 hours, if they are immersed in one percent solution of complexon-3 after cleaning. This favorable effect of complexon-3 can be explained by the blocking of activity of tyrosinase or other related redoxidases. It is known that the darkening of cleaned raw potatoes is related to the transformation of tyrosine into melanine under the influence of tyrosinase. This reaction of tyrosine-melanine is eliminated in the presence of complexon-3.

On the basis of these considerations, it would be possible to utilize complexons for the elimination of potato-darkening in cooking. It is most probable that this darkening can be prevented by adding complexon-3 to the water in

which potatoes are cooked, or by processing potatoes in a water solution of complexon-3 prior to the steam action. Thus, complexon-3 may possibly be utilized to eliminate undesirable influences of metals and metal-containing enzymes in food technology.

However, utilization of complexons, in food technology, has not been permitted up to the present throughout the world.

This situation is due to the fact that to the present time the effect of these substances on the human organism and on various processes taking place in it has not been thoroughly investigated. Therefore, despite the fact that many authors recommend complexon-3 in wine filtration, other authors (Seris, 1954) point out that the utilization of complexon in wine filtration is not permitted by law. Many assert that though the complexons are useful in wine filtration, they are nevertheless foreign substances, whose effect on the organism had not been studied and, therefore, their employment can not be recommended for the present (Deibner and Bouzigues, 1954).

Therefore, the problems of the biological effect of complexons are closely connected with their utilization in food technology. These problems have been very inadequately studied up to the present time. Thus, a number of authors (Teisinger and Srbova, 1956; Sandi, 1955; Hunzinger and Ortelli, 1954) point out that there is no convincing proof in the literature on the positive or negative effect of complexons on the metabolism of mineral substances on the organism. The same can be said of the relation of complexons to the metabolism of other vitally important substances.

In 1951 (Child, 1951) attention was for the first called to the strong activity of complexons in binding micro- and macro-elements under experimental conditions. It was observed in experiments on rats with polycythemia caused by 0.1 percent of cobalt solution that a 0.5 percent solution of complexon-3 can inhibit, and a 0.2 percent solution reduce the development of polycythemia. In 1952, attention was called to the great activity of complexons in binding lead (Rubin and Foremann, Bauer and associates; cited according to Tefsinger and associates, 1956), and in 1953 in binding iron (Wishinsky and associates, 1953) and calcium (Berzin, 1953).

Subsequently, the strong activity of complexons in binding other elements in vivo was also demonstrated (D. I. Semenov and I. P. Tregubenko, 1958; Hart and associates, 1955; Millar and associates, 1954; Odescalchi and Scudier, 1956; Rieders and Birger, 1956; Scudier and Tinazzi, 1956). These investigations clarify the problems of the

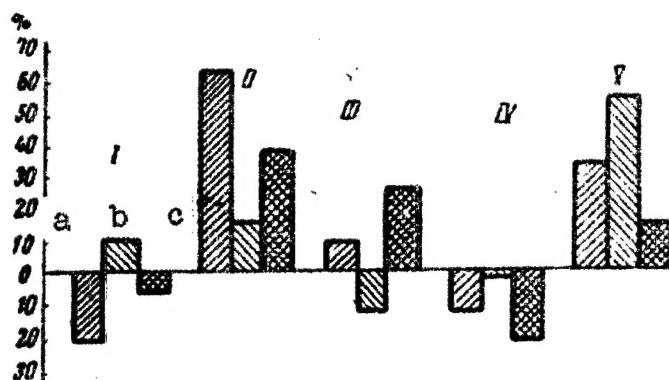
effect of complexons on the metabolism of mineral substances under pathological conditions, i. e., poisoning with lead or other metals in hemosiderosis, radiation sickness, etc.

We found only one report (Rieders, 1955) concerning the relation of complexons to the metabolism of mineral substances under physiological conditions. The author of this work experimented on rats which had received food containing 0.2 percent solution of calcium salt of complexon-3, and water containing 0.1 percent of complexon-3 and during four months of observation found no difference in weight, number of erythrocytes, and hemoglobin in the experimental and control animals. However, after these animals had been killed, considerable hemosiderous deposits were detected in two out of five males and four out of five females. Regarding the content and distribution of iron, copper, tin, and lead, definite differences were observed in the tissues of the experimental and control animals. The author explains these phenomena by the fact that the calcium salt of complexon-3, following peroral administration, inhibits the above mentioned ions in the digestive tract, thus exerting a negative effect on their metabolism. This work represents the only contribution, and its great defect is that conclusions are made on the mean figures based on five animals. The data also are not worked out statistically.

In our aim to contribute to the clarification of the biological effect of complexons, we decided to undertake, parallel with other investigations, the study of the relation of complexon-3 to the metabolism and to ion distribution in the organism (Vozar, 1957). During the study of the metabolism of mineral substances, we were at first interested in the changes of their level in tissues and fluids of the organisms following oral administration of complexon-3. Later, we supplemented our experiments with some special studies. We tried to ascertain whether the administration of complexon-3 would have an effect on the metabolism of various substances and, if so, to what extent will these changes be reversible after the cessation of its administration.

The experimental studies were conducted on groups of male rats of the Wistar species and on female guinea pigs. We have cited a detailed description of our experiments and methods in our previous publications. In this article we shall summarize only certain basic data obtained in the study of the effect of complexon-3 on a live organism.

In investigating the effect of complexon-3 on the metabolism of mineral substances, we found serious disturbances in the metabolism of copper, iron, and phosphorus. The level of these ions in the tissues of animals which had received complexon-3 per os (24-hour dose was 40 mg per 100 gm weight)



Changes in the level of copper (a), iron (b), and phosphorus (c) in tissues of rats following administration of complexon-3 for a period of 20 days with a daily dosage of 40 mg per 100 gm weight.

I -- liver; II -- cerebrum; III -- spleen; IV -- kidneys; V -- muscles.

was different from that of the control group (see Fig.). Complexon-3 causes mainly a definite accumulation of copper, iron, and phosphorus in the cerebrum and skeletal musculature. It has been proven that the rise in the level of ions in the cerebrum and skeletal musculature represents a definite and statistically fully corroborated phenomenon. Thus, for example, the level of copper in the cerebrum of experimental rats which had received complexon-3 for a period of 20 days (as compared to the level of controlled rats) was higher by 62.7 percent ($P = 0.05$), the level of iron by 10.9 percent, the level of phosphorus by 34.2 percent. In the skeletal musculature the copper level was higher by 32.4 percent ($P = 0.05$), iron by 56.7 percent ($P = 0.001$), and phosphorus by 15.4 percent ($P = 0.05$). Other tissues which we had investigated reacted variously to the action of complexon-3. We observed a definite reduction in the quantity of copper in the liver (22.4 percent, $P = 0.002$) and insignificant changes of the iron and phosphorus levels. A definite accumulation of phosphorus was noted in the spleen (24.6 percent, $P = 0.05$), a reduced content of copper in the kidneys (14 percent, $P = 0.01$) and phosphorus (23 percent, $P = 0.05$). Analogous results were observed in experiments on rats who had been receiv-

ing complexon for a period of 40 days. In this case we observed in the animal tissues a definite rise in the concentration of copper (55.3 percent, $P = 0.001$), iron (99.8 percent, $P = 0.001$), phosphorus (13.6 percent, $P = 0.001$) in the skeletal musculature, and a higher concentration of iron (28.3 percent, $P = 0.001$) and phosphorus (7.5 percent, $P = 0.05$) in the liver. We noted simultaneously a statistically definite reduction of copper concentration in the spleen (27.8 percent, $P = 0.001$). Thus, the observations of this series of experiments confirmed the results of our previous investigations.

Special experiments showed that changes in the distribution of copper, iron, and phosphorus caused by the administration of complexon-3 continue also after the cessation of administration. Thus, it was established that the concentration of copper (38.1 percent, $P = 0.001$) and iron (41.6 percent, $P = 0.001$) remains higher in the skeletal musculature; at the same time there is a higher concentration of copper (41.6 percent, $P = 0.001$) in the kidneys, and iron (15.4 percent, $P = 0.001$) in the spleen. Higher level of copper in the cerebrum (39.8 percent, $P = 0.001$) remains unchanged even after cessation of complexon-3 administration.

The results of study of the balance of separate elements also indicate the intensive interference of complexon-3 in the metabolism of mineral substances. The mean figures of 20-day balance tests (Table 1) show that animals, who had received orally complexon-3, eliminate with feces 115.4 percent more copper than the controls.

This effect of the action of complexon-3 is manifested in the reduced retention of copper. We observed a reverse tendency in studying the balance of iron and phosphorus. In rats, who had received complexon-3, the retention of iron increases approximately threefold, and the retention of phosphorus twofold; when this occurs, the elimination of iron via urine and feces is reduced, as is the elimination of phosphorus via feces.

In comparing results obtained in studying iron-phosphorous balance with the results characterizing the level of corresponding ions in tissues, we must state that complexon-3 impairs the physiological sequence of biochemical reactions in the organism leading to serious displacements of micro- and macro-elements in the tissues. Peroral administration of complexon-3, thus, induces pathological changes in the distribution of ions in tissues.

The character of these changes, as confirmed by our observations, consists not only in the elimination of cations of metals from the organism, but also, primarily, their non-physiological concentration. These changes occur occasion-

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Table 1

Mean figures of intake, elimination (via urine and feces), and balance of copper, iron, and phosphorus of rats of the control group and of rats which had received complexon-3 daily for 20 days in the amount of 40 mg per 100 gm weight (the quantities of copper and iron are indicated in gammas, phosphorus in mg.)

Name of element	Group	No. of rats	Elimination				Balance
			In- take	Urine	Feces	Total	
Copper	Control	5	86.4	11.8	22.7	34.5	+51.0
	Experimental	5	84.9	10.5	48.9	59.4	+25.5
Iron	Control	5	699.3	68.4	704.7	773.1	-73.8
	Experimental	5	688.6	62.1	589.5	651.6	+37.0
Phosphorus	Control	5	77.6	1.0	72.0	73.0	+4.6
	Experimental	5	75.7	2.9	61.8	64.7	+11.0

ally, despite their increased elimination, and resemble for example, the accumulation of copper in the cerebrum and skeletal musculature in poisoning caused by copper derivatives (P. V. Rodionov; cited according to A. O. Voynar, 1953). From our results we can assume that metabolic disturbances of mineral substances under the effect of complexon-3 are also related to the binding of some ions with complexon-3 in the digestive tract. These ions therefore do not participate in the normal physiological processes, and, having combined with complexon-3, are carried by the flow of blood into various organs and tissues. This concept of ours is in accord with the observations of other authors (Rieders, 1955), who have demonstrated in experiments on an isolated intestinal loop that complex compounds of complexon-3 with copper or iron, as well as with other cations, easily pass through the intestinal wall. These compounds may disintegrate in the circulatory system, and this condition, in turn, may lead to such pathological changes as hemosiderosis, etc. Even if our observations in this respect required supplementary special studies, it still seems to us that the presence of an enhanced content of iron in some tissues, following the cessation of complexon-3 intake, indicates the presence of hemosiderous nidi. We tend to this conclusion, on the one hand, because it is known that iron in these nidi is present in

a tri-valent form, and, on the other hand, because the tri-valent iron may possibly be carried into the circulatory system following an oral administration of complexon-3. Changes noted in phosphorus metabolism, it seems to us, are caused by the impairment of the calcium-phosphorus balance induced by the reaction between complexon-3 and calcium.

In view of the serious metabolic disturbances of mineral substances observed in rats after oral administration of complexon-3, and in view of subsequent information obtained by us on vitamin C distribution in the tissues of rats and guinea pigs which had received complexon-3 (Vozar, 1958), we decided to undertake further study of its biological effects. We based these studies on the data which suggested that the distribution of vitamin C in various animal tissues may serve as an indicator of the state of the organism (M. F. Merezhin-skiy and L. S. Cherkasova, 1956; B. A. Lavrov and B. I. Yanov-skaya, 1956; Sayers and associates, 1948); in our experiment this distribution may serve to indicate the metabolic changes of mineral substances induced by complexon-3. Since the cortex-hypophysis-suprarenals system participates in these processes, and inasmuch as we observed an intensive rise of the content of copper in the cerebrum, as well as a considerable discharge of vitamin C, we tried to ascertain whether these changes of copper concentration in the cerebrum involve predominantly the gray matter of the cortex. We established in corresponding experiments (Table 2) that the rise in the copper level in the brain takes place chiefly in the cortical gray matter.

Table 2

The Mean Content of Copper in the Cerebrum of Rats After a 20-Day Administration of 40 mg of complexon-3 per 100 gm weight

Group of rats	Number of rats	Cerebral gray matter (mg %)	Average error	Extra-cortical gray matter (mg %)	Average error
Control	13	2,078	0.367	3.845	0.909
Experi-mental	14	4.506	0.877	2.432	0.468

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The rise of 116.8 percent in the level of copper in this section of the cerebrum was statistically significant ($P = 0.05$). On the basis of these data we suggested an hypothesis that complexon-3 induces a system of reactions the first of which concerns the effect of complexon-3 on the metal (thus excluding certain important enzymic processes) leading to the damage to the gray matter of the cerebral cortex. Subsequently impulses are carried from here to the hypophysis which in turn transmits them to the suprarenals. We feel, therefore, that the effect of complexon-3 on the metabolic processes in the organism is incomparably more extensive than had been thought originally.

Such a hypothesis of the mechanism of the complexon-3 action seems to us to be in complete accordance with our subsequent observations. Of special interest in this respect are the data that oral administration of complexon-3 causes a reduction of serum gamma-globulin with a simultaneous increase of non-protein nitrogen in the serum. We noted such effect of complexon-3 in experiments on rats and guinea pigs. Concerning our subsequent observations in this respect, the data relating to the blood picture in rats deserve attention. It has been established that complexon-3 causes a reduction in the hemoglobin as well as changes in the picture of the white blood, manifested in the reduced number of leucocytes. These data support the conclusion that oral administration of complexon-3 causes metabolic disturbances in a living organism.

From the above observations, we must conclude that complexon-3 is undesirable for nutrition from the hygienic point of view, since it causes disturbances in vitally important organic processes. Therefore, complexon-3 can not be recommended for the technological preparation of food products designed for human nutrition.

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